The Pathological Relationship Between the Host and Parasite in Varieties and Strains of Watermelons Resistant to *Fusarium Niveum* E. F. S.

By Joseph Jay Wilson

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IOWA STATE COLLEGE OF AGRICULTURE
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SUMMARY

Watermelon plants, at all stages of growth, are liable to attack by *Fusarium niveum* which enters through the root tips and ruptures formed by newly developed lateral roots.

Epidermal cells of the zones of elongation and maturation at the side of the root 2 to 6 mm. back of the zone of meristematic activity were as readily penetrated as meristematic tissues at the tip. After penetrating the epidermis, hyphae continued intracellularly through the cortical cells to the pericycle where the massed mycelium broke down the endodermal cell walls and entered the xylem vessels.

Entrance of the pathogene through the ruptures formed by lateral roots was accompanied by the formation of lesions, intracellular penetration of the cortical and parenchymatous tissues, and disorganization of the endodermis by the action of massed hyphae, which then entered the xylem vessels.

Infection of seedlings at or shortly after germination of the seed, combined with favorable conditions for the pathogene led to rapid invasion of the xylem vessels with mycelium in the primary root, accompanied by a high percentage of wilting.

Older plants, repeatedly infected through young lateral roots, apparently succumbed from a series of internal pathological disturbances involving the accumulation of gum-like materials, tyloses and mycelium in the xylem vessels, particularly those of the primary root where the conductive capacity of the vessels was so reduced in time that wilting ensued.

Gum-like materials and tyloses in the xylem of diseased plants seem to be produced by living cells of the host, injured by either toxic, metabolic or enzymatic products of the wilt pathogene.

Older resistant plants seemingly withstood attacks of the wilt pathogene, which in seedlings often proved fatal. Apparently no well developed defense mechanism had time to develop and function in the early seedling stage. It was significant that resistant plants, which survived in fields heavily infested with *Fusarium niveum* had bands of gum-like material surrounding the older xylem near the center of the root axis, while the secondary xylem at the periphery of the stele remained unaffected. On the other hand, wilted susceptible plants were filled with gum-like materials throughout the primary root xylem. Neither resistant nor susceptible plants, grown in soils free of the wilt pathogene, had appreciable quantities of gum-like materials and tyloses in the primary root xylem.

Greenhouse indexing of resistant seedlings has proved of value in the Iowa Belle and Pride of Muscatine varieties, but,

with the dosage of inoculum employed, Iowa King selections failed to show measurable differences in resistance when compared with susceptible checks.

In 4 years, field studies on soils heavily infested with the wilt pathogene, resistant plants seemed more resistant with age, the heaviest mortality being within the first 16 to 24 days, after which few plants wilted; susceptible checks continued to wilt throughout the season.

Changes in air and soil temperatures and precipitation retarded or accelerated wilting for periods of 1 to several days; however, irrespective of environmental factors, there was an upward trend in the percentage average daily wilt, which reached a maximum at 23 to 39 days after planting susceptible seedlings and at 16 to 24 days with resistant seedlings.

Pride of Muscatine, Iowa King and Iowa Belle have proved to be suitable stock for the transmission of resistance, the last named variety being especially desirable as a resistant parent. Backcrossing the F_1 hybrid (resistant x susceptible variety) to the resistant parent has proved the most effective method of building up resistance when susceptible and resistant lines were involved.

The Pathological Relationship Between the Host and Parasite in Varieties and Strains of Watermelons Resistant to *Fusarium Niveum* E. F. S.[1]

By Joseph Jay Wilson[2]

By 1924 a wilt disease caused by *Fusarium niveum* E.F.S. practically had eliminated commercial production of watermelons in the large sandy land areas of southeastern Iowa. This disease had become a menace also in lesser areas of the state. Efforts initiated in 1925 and continued since by the Iowa Agricultural Experiment Station to rehabilitate the industry were motivated by the economic distress of the farmers in these areas. The beginning phases in this station's investigation of the disease [Porter (47) and Porter and Melhus (53)] have dealt with the biology and pathogenicity of the organism; the conditions and symptoms surrounding infection; methods for control; and the successful isolation of wilt-resistant watermelon selections and hybrids.

During the course of the breeding program, each generation added greater numbers of samples to be tested for resistance. This in turn required larger tracts of land, and handling of many selections later was found undesirable because of their susceptibility to *Fusarium niveum.* It was highly desirable that a greenhouse indexing test be substituted for the field test. Such a technique was devised by Porter and Melhus (53), but in certain selections the results of the greenhouse indexing test were not sustained by subsequent field tests. It was necessary to determine the cause of this variability before further indexing could have real value in predicting the field response of different selections.

When resistant selections were grown in field plots heavily infested with *Fusarium niveum,* it was noted that the death rate was higher in the seedlings than in the older plants. After the seedling stage, a high percentage survived throughout the remainder of the season. This high mortality in seedlings could not be explained by a susceptible segregation, since many of the resistant selections were the progeny of lines inbred four to six generations and grown in fields heavily infested with the wilt pathogene.

[1] Taken from a thesis submitted to the faculty of the Graduate College, Iowa State College, in partial fulfillment of the requirements for the degree, doctor of philosophy. Project No. 71 of the Iowa Agricultural Experiment Station.

[2] The author expresses his deep appreciation for the direction and guidance given by Dr. I. E. Melhus throughout the course of this study and of the helpful assistance from Dr. J. E. Sass in histological preparations.

Before further progress could be made on the development of resistant varieties, it became necessary to learn more about infection and what occurred in the host after the pathogene was established. The results of such a study and their application in the interpretation of greenhouse and field experiments are presented in this paper.

METHOD OF INFECTION

Infection studies with *Fusarium niveum* were reported first by Smith (61, 62), who demonstrated the pathogene to be soil-borne and thought infection of the host took place principally when plants were young. He observed that the root system of the watermelon plant was first to be invaded, but he did not find how the pathogene gained entrance. Orton (41) noted the presence of small tufts of roots at suspected invasion points on lateral roots of watermelon plants infected with the wilt pathogene. Taubenhaus (64) thought that infection sometimes occurred from leaf and stem wounds made by the striped cucumber beetle, *Diabrotica vittata* Fab., which he found to carry the wilt pathogene externally. Porter (47) interpreted his experiments to indicate that the wilt pathogene probably entered the host naturally through root hairs and the epidermis of the hypocotyl. He also studied externally the root systems of many plants growing in infested soil, observed numerous lesions on wilted and healthy appearing plants and isolated the wilt pathogene from the lesions and root crowns.

Yoshii (75) more recently has described the invasion of *Fusarium niveum* in the meristematic region of the root tips of very young watermelon seedlings when cultured with the pathogene on an agar agar medium in petri dishes. He decided that infection was successful only when the host was very young and might well be called "meristematic infection" and not "cuticular infection." Yoshii (76) (77) thought that internal parts of the plant more remote from the original point of invasion became infected by microconidia which migrated in the transpiration stream of the xylem vessels.

Diverse methods of host infection by other parasitic vascular fusaria have been reported. Variation in certain instances might be attributed to the conditions under which the studies were conducted and the scope of the observations. Bolley (5) illustrated the infection of young flax roots by *Fusarium lini* Boll. as direct penetration of the epidermal cells. Tisdale (66) later described hyphal invasion of root hairs, stomatal openings and epidermal cells of flax seedlings cultured in test tubes. He observed that the progress of the hyphae was temporarily obstructed by the suberization of newly formed cambium cells in resistant varieties of flax.

Brandes (6) found that *Fusarium cubense* (E.F.S.) Br. easily invaded meristematic tissue at the tip of young, fleshy banana roots, the epidermal and cortical cells being deeply penetrated, but that the root hairs were not attacked. Wardlaw (70) working with the same pathogene, observed that suberized cambiform-cells were formed by the host in advance of the invading hyphae.

Smith and Walker (63) showed that *Fusarium conglutinans* Woll. penetrated intercellularly and occasionally intracellularly the growing point of young cabbage roots, but that the root hairs were seldom invaded. Homozygous, resistant cabbage strains were penetrated only occasionally and when this happened the morphological reaction was very similar to that found in susceptible plants.

Rosen(54) observed a rotting of the tissue about the natural wounds of extruded secondary roots when the cotton plant was attacked by *Fusarium vasinfectum* Atk. These roots were most subject to attack when succulent. Dharamajula (11), working with the same pathogene, cultured cotton seedlings in a soil medium and found that the outer epidermal cells of the radical were penetrated directly by the hypha of the germinating conidia after which the hyphae continued intracellularly through the other tissues until they reached the xylem. Invaded cells of resistant and susceptible seedlings apparently were uninjured and did not show any consistent differences in reaction toward the pathogene. Fahmy (14) found that *Fusarium vasinfectum* var. *aegyptiacum* T. Fahmy penetrated the epidermal cells more readily at the sides of the root cap than at the apex of young cotton seedlings when cultured with the pathogene on agar agar media in test tubes. The pathogene destroyed the host tissues, continued its invasion and became established in the vascular bundles.

Edgerton and Moreland (12) did not determine the exact method by which *Fusarium lycopersici* Sacc. infected tomato plants, but found that "in seedbed plants the discolored bundles could be readily traced down to a small dead root or a discolored area on a root."

The frequency of lesions on the basal part of lateral roots of wilted watermelon-seedlings suggested that this symptom might be intimately associated with primary infection of the host by *Fusarium niveum.* For this reason a study of the root lesions on young, infected watermelon-seedlings was made under controlled conditions.

MATERIALS AND METHODS

Seedlings of a highly susceptible inbred strain of the variety, Jugo Slavia 7, and F_5 progeny of the wilt-resistant variety, Iowa Belle, were selected for a study of root lesions. Seeds of each were surface sterilized with mercuric chloride (1-1000), aseptically washed and germinated at 25° C. on moist blotting paper

in petri dishes. When the radicals were about 2 cm. long and previous to the emergence of secondary roots, the seedlings were transferred to crystalizing dishes containing steamed quartz sand. A heavy spore suspension of *Fusarium niveum* was introduced into one series of sand cultures before the seedlings were

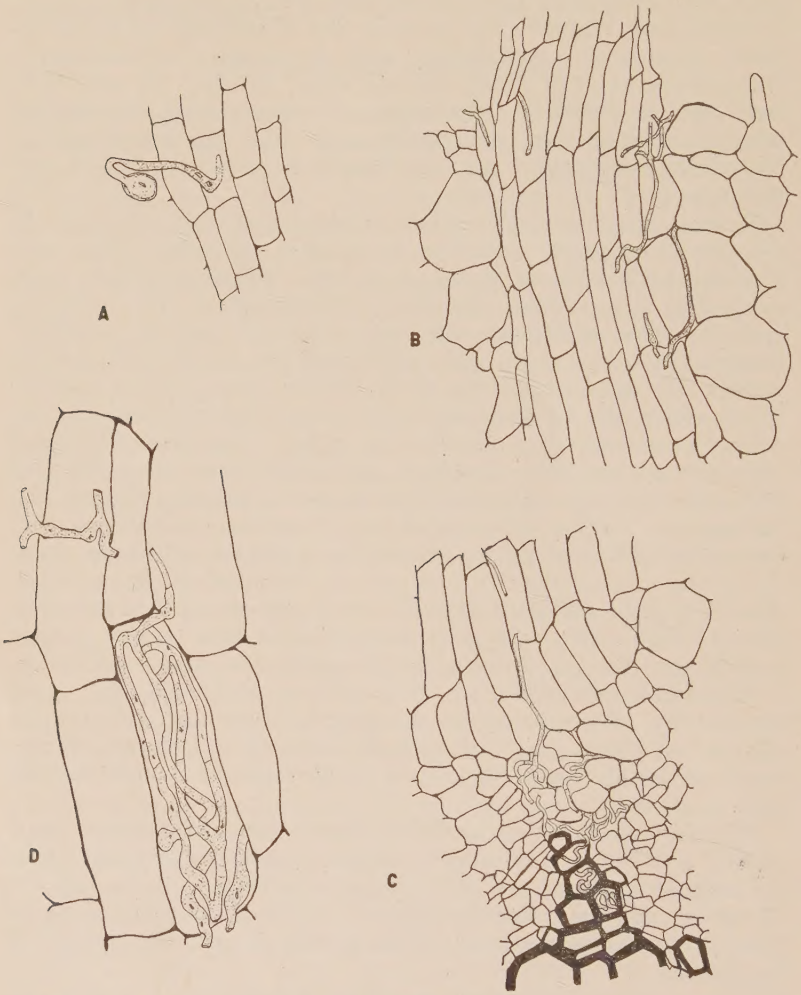


Fig. 1. Lesion and invasion of pathogene at secondary root rupture of seedling shown. A. Germinated conidia of *Fusarium niveum*. Hypha penetrating directly through an epidermal cell of a secondary root. X370. B. Transverse section of 6-day-old seedling primary root and tangential section of the secondary root, showing a necrosis in tissues at its base and mycelium invading. Note mycelium also along root rupture. X190. C. Transverse section of an 8-day-old seedling primary root. Mycelium breaking down cells at the border of the pericycle and established in the younger cells of the xylem ray of a primary root. X190. D. Mycelium coiled in a parenchyma cell of a secondary root. Hyphae have swollen and pressed through the cell walls. X560.

planted. Spore suspensions of the pathogene were secured directly from plate isolations made of root tissues from wilted watermelon seedlings. White quartz sand precluded soil stains and separated readily from the roots. The food reserve in the cotyledons proved sufficient for the seedlings during the short period that they were grown. Two wilt-free and two wilt-infested sand cultures, each including six plants (three susceptible and three resistant), were examined at 48, 72- and 144-hour periods. All cultures were kept moderately moist and held at 20° to 25° C.

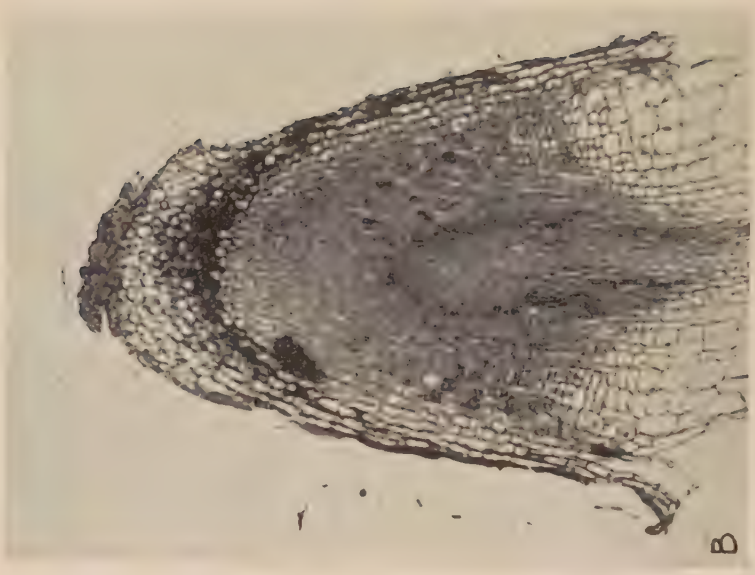
When floated with water in a petri dish cover, the root systems could be viewed clearly at a magnification of 50 diameters. Free-hand sections stained with a dilute solution of lacto-phenol (10) combined with cotton-blue afforded a satisfactory means for detecting mycelium in preliminary observations. More detailed histological studies were made from permanent mounts of tissues killed in chrome-acetic-formalin solution (27 cc. of 1 percent chromic acid, 68 cc. of 1 percent acetic acid, and 5 cc. of 34 percent formalin). Paraffin sections were cut 10, 13 and 15 microns thick, stained with Meyer's hemalum (57) and counter-stained with safranin.

LESIONS ON SEEDLING ROOTS

The first appearance of any pathological activity was observed after 72 hours when examination was made of paraffin sections of seedling roots from sand cultures infested with the wilt pathogene. In one instance, the hypha of a germinating conidium had penetrated the epidermal cell-wall of a lateral root (fig. 1, A). Previous to this time, free-hand and paraffin sections of root bases and tips appeared to be free from any fungal growth.

Seedlings, which had been cultured for 144 hours in sand infested with the wilt pathogene, had present light brown lesions at the base of some lateral roots. Occasionally young lateral roots (0.5 cm. long) were completely discolored. Such roots had become infected at or soon after their emergence. Usually lesions occurred on one side only and at the base of a lateral root. In a few instances, however, the root was completely encircled. Beyond the point where the lesion had developed the root tissue appeared healthy.

There were marked pathological disturbances at points where lesions developed. Epidermal cells were partially collapsed, and the protoplasm of the cortical cells had become discolored. Mycelium was scattered throughout the lesion (fig 1, B). The progress of the pathogene was easily traced in paraffin sections. Hyphae of the fungus penetrated the epidermis irrespective of root hairs and progressed intercellularly and intracellularly through the cortical and stellar tissues directly to the xylem. Upon reaching the xylem of the lateral root the mycel-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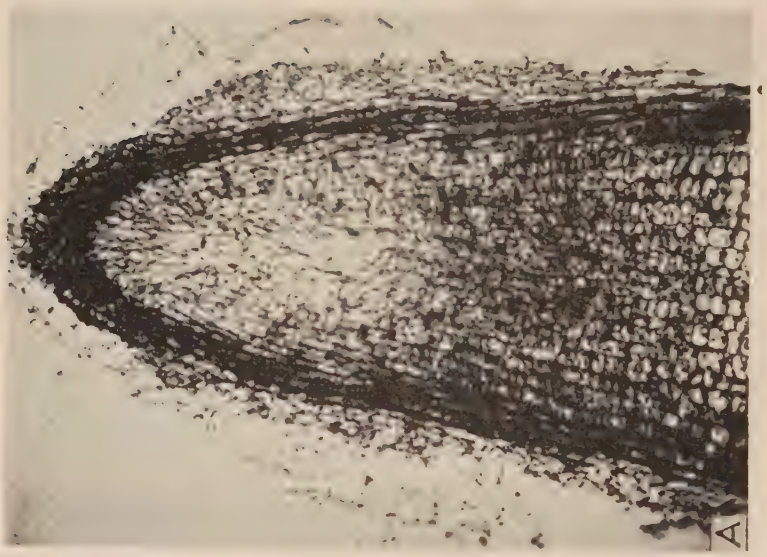
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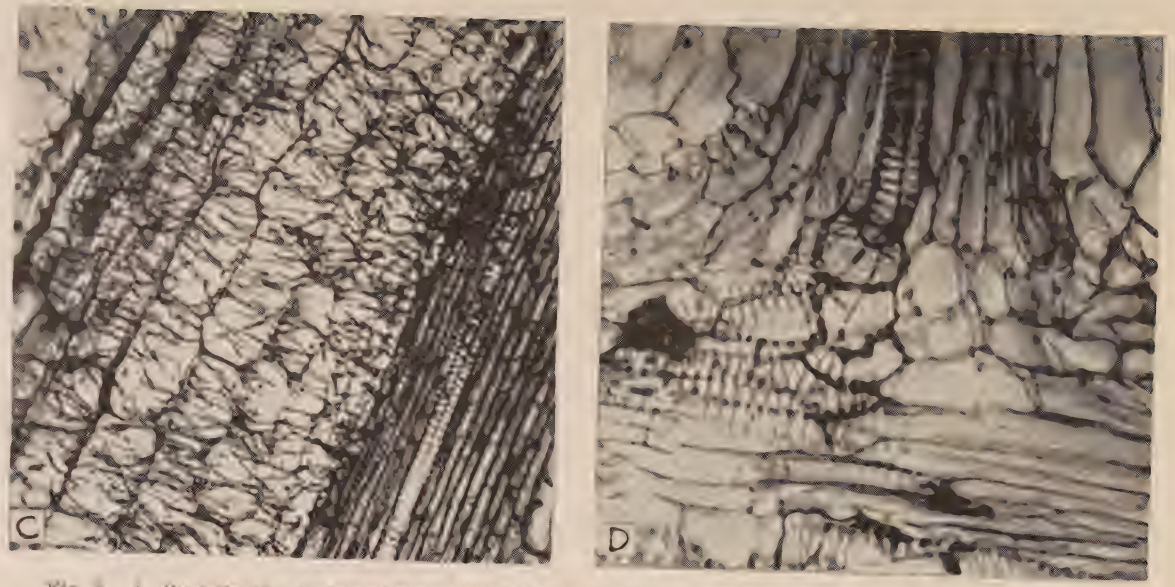


FIG. 2. A. Root tip of a watermelon seedling 24 hours after contact with a *Fusarium niveum* colony cultured on potato dextrose agar in a petri dish. Note complete invasion at the root cap and sides of the root tip. X88. B. Root tip of a watermelon seedling 24 hours after contact with *Fusarium conglutinans* cultured on potato dextrose agar in a petri dish. Only slight invasion has occurred. X88. C. Longitudinal section cut in series of the root tip shown in A. Invasion of mycelium from the side of the root directly to the tracheal vessels. X184. D. Longitudinal section of a secondary and a primary root showing mycelium entering a tracheal vessel of the latter from the invaded trachea of the former. Plant 10 days old. X355.

ium moved up and down and was found in the trachea of the primary root at the junction of the xylem vessels (fig. 2, D).

Hyphae advanced through the rupture made by the secondary root, but more interference was encountered and their progress was not so rapid. Mycelium was sometimes found coiled in the more mature parenchyma cells (fig. 1, D). The heavier walled cells of the endodermis were penetrated only after considerable mycelium had accumulated at the border of the pericycle. Cell walls were broken down at this point and the cavity was filled with hyphae (fig. 1, C). The cells of the host were destroyed when the mycelium became abundant. Necrosis of the host tissues was contingent upon an accumulation of the fungal hyphae. Fewer lesions appeared on roots of resistant seedlings and certain differences were noted in the progress of the pathogene after gaining entrance to the xylem. These observations will be discussed in the study of the internal pathology of the host.

ROOT TIP INVASION

Complete necrosis of young lateral roots, 0.5 to 1.0 cm. in length, on wilted seedlings 12 days old, indicated that infection had occurred early in their development. It was thought advisable to investigate the infection of root tips by repeating the study made by Yoshii (75), who stated that "*Fusarium niveum* invades first the root-cap and then into the primordial meristem or directly attacks the primary meristem of the host plant." Seedlings with radicals about 1.5 cm. long were used for the experiment. The tips of seedling radicals were placed in contact with colonies of the organism cultured on potato dextrose agar in petri dishes. After being incubated for 24 hours at 25° C. mycelium had grown around the root tips and along the side for a distance of 6 mm. or more. The tips were discolored similarly to infected lateral roots of older seedlings. Tangential, longitudinal and transverse sections of the infected root tips were examined. Hyphae completely permeated the root-cap and meristematic tissues of the tip but infection was not limited to these parts. Epidermal cells at the side of the radical 3 to 6 mm. back of the zone of meristematic activity were penetrated by hyphae, which progressed intracellularly through the cortical and stellar tissues and entered the young xylem vessels by a direct radial route from the surface of the root (fig. 2, C). Upon reaching the endodermis the hyphae of the pathogene penetrated less readily, but after accumulating at any particular point, the cell walls were destroyed. Susceptible and resistant seedlings seemed to be invaded with equal ease under the conditions of the experiment.

Infection under such an artificial situation as in the above experiment may be open to question as Young (78) has shown. For this reason the infection of watermelon seedlings by other vascular-fusarial pathogenes was attempted in conjunction with the

above study. Root-tips of watermelon seedlings were exposed to the attack of *Fusarium conglutinans, Fusarium lycopersici* and *Fusarium lini* for the same period of time and under the same conditions. None of these organisms exhibited the degree of infectivity shown by the watermelon wilt organism. The root tips were covered with mycelium which destroyed the cells of the root cap, but the remainder of the tip was not invaded (fig. 2, B). Further invasion seemed to be arrested by the reaction of the host cells whose walls stained deeply with safranin. Hyphae were abundant throughout this superficial lesion but did not penetrate inwardly more than a few cells from the surface.

EXTERNAL AND INTERNAL SYMPTOMS OF HOST

External symptoms developed by watermelon plants, when attacked by *Fusarium niveum,* are contingent upon the nature and degree of infection. Abrupt wilting of plants and damping-off of seedlings were first described by Smith (61). To these symptoms, Porter (47) and Porter and Melhus (53) have added seedling-rot, root canker, stunting and yellowing.

The internal symptoms of the diseased plants have not been studied in as great detail as the external symptoms. Smith (62) observed the hyphae of *Fusarium niveum* more or less completely filling the xylem in the stem and root of wilted plants. In later stages of wilting the xylem was darkly stained and mycelium extended into the parenchyma and other tissues near the xylem. Porter (47) isolated the pathogene from all parts of wilted plants and found that the tissues were destroyed completely in the case of seedling rot. Sleeth (59) reported tyloses to be abundant in the xylem of 1- to 4-months-old plants infected with *Fusarium niveum.* He believed that tyloses either preceded or coincided with the spread of the pathogene.

Underground parts of the watermelon plant have been shown to be the initial points of attack by *Fusarium niveum,* and root lesions have been shown to be associated with primary infection. Since the internal symptoms produced by the pathogene in the seedling are modified in the older plant, further study of the external and internal pathology of the host will be divided into two parts: seedlings and older plants.

SYMPTOMS ON SEEDLINGS

Watermelon seedlings wilt in large numbers during the first few weeks after emergence, when grown in field soils heavily infested with *Fusarium niveum.* A similar mortality occurs under greenhouse conditions if the soil is heavily dosed with inoculum (47) (53). In either situation frequent and early infection of the plant is possible. Smith (62) reported 81 days to be the longest and about six days the shortest incubation period of the

organism observed by him in plants grown in soil infested with *Fusarium niveum.*

EXTERNAL SYMPTOMS

In young seedlings the first symptom of disease commonly noticed is a slight drooping of the cotyledons. This is followed in a few hours or a day, depending upon the relative humidity of the air, by an increased flaccidity and later a complete wilting of the cotyledons.

Extensive necrosis of the roots seldom occurs in seedling wilt. Hundreds of wilted seedlings taken from the field or greenhouse have shown no apparent reduction in root development from the presence of the organism. Roots of wilted seedlings were studied by an experiment in which plants were grown in steamed soil infested with a pure culture of *Fusarium niveum* and held at 22° to 25° C. Root systems of wilted plants, in the cotyledonary leaf stage, were washed free from the soil and compared with those of healthy plants grown in steamed soil. Those of wilted seedlings were as well developed and showed no apparent deleterious growth effect from the presence of pathogene. There were, however, yellowish brown lesions encircling the base of certain lateral roots (fig. 3).

Fig. 3. Lesion at base of a secondary root of a wilted seedling, 20 days old, grown in soil infested with the wilt pathogene.

INTERNAL SYMPTOMS

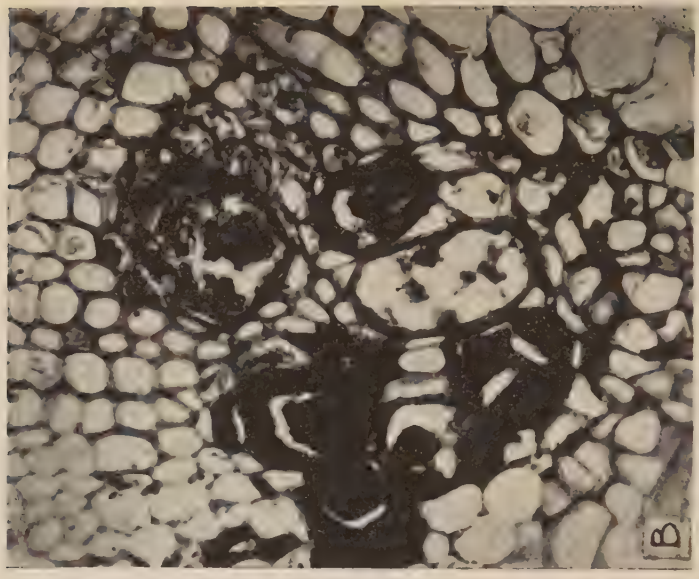
The xylem vessels are seldom discolored in young plants which have wilted. When transverse or longitudinal sections are cut in series from the primary roots, a region at about 2 cm. below the base of the hypocotyl is found in which the xylem vessels are completely filled with mycelium (fig. 4, C). When sections are cut above and below this particular zone, complete plugging is less frequent. Vessels in one xylem ray of the tetrarch are often more congested with mycelium than are the others. This suggests that the pathogene may have invaded through a lateral root, since these develop from and have direct vascular continuity with the anterior points of the xylem rays.

The xylem vessels in the hypocotyl of seedlings are seldom completely congested with mycelium. Sections cut in series from the hypocotyl at the base show a diminishing quantity of mycelium present in the xylem as the distance increases upward. Usually the vessels in adjacent vascular bundles or those on one side of a hypocotyl contain more mycelium than do the others. Cells bordering the invaded trachea and tracheids do not appear disturbed. The pathogene limits itself chiefly to the xylem (fig. 5, E).

Upon reaching an end wall of a xylem cell the mycelium coils in a dense mass from which a few filaments continue through the pits of the end wall into the lumen of the adjoining cell. Similar penetration occurs through the pits of side walls, when an occasional filament passes into an adjacent xylem vessel.

Mycelial congestion of the xylem vessels in the primary root is the most prominent feature in the internal pathology of wilted seedlings. Apparently the pathogene multiplies so rapidly and in such abundance in the primary xylem of the young primary root that the functional activity of the vessels is decreased to a point where wilting of the seedling occurs. Whether there are accessory influences which induce wilting, such as metabolic products or toxins of the pathogene, may be questioned. Young plants, in the first stages of wilting, regain their turgescence and continue to live for a week or more when placed in a moist chamber and held at varying temperatures, 18° C. and 25° C. (fig. 6). Further symptoms of wilting do not appear when the plants are kept in the moist chamber. After 3 to 7 days water-soaked lesions develop on the hypocotyls of plants which have previously shown symptoms of wilting. These lesions are usually at the base of the hypocotyl near the surface of the soil. Pure cultures of *Fusarium niveum* were isolated from the host tissue of the lesions. When sections were cut from this tissue, the pathogene was found emerging from the xylem vessels and ramifying through the surrounding cortical tissues.

Recovery from wilting by diseased seedlings in an environment where transpiration is greatly reduced indicates that a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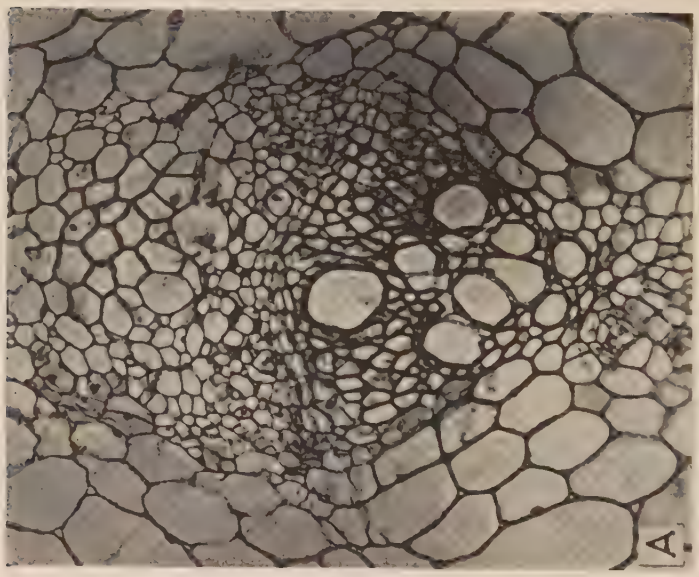
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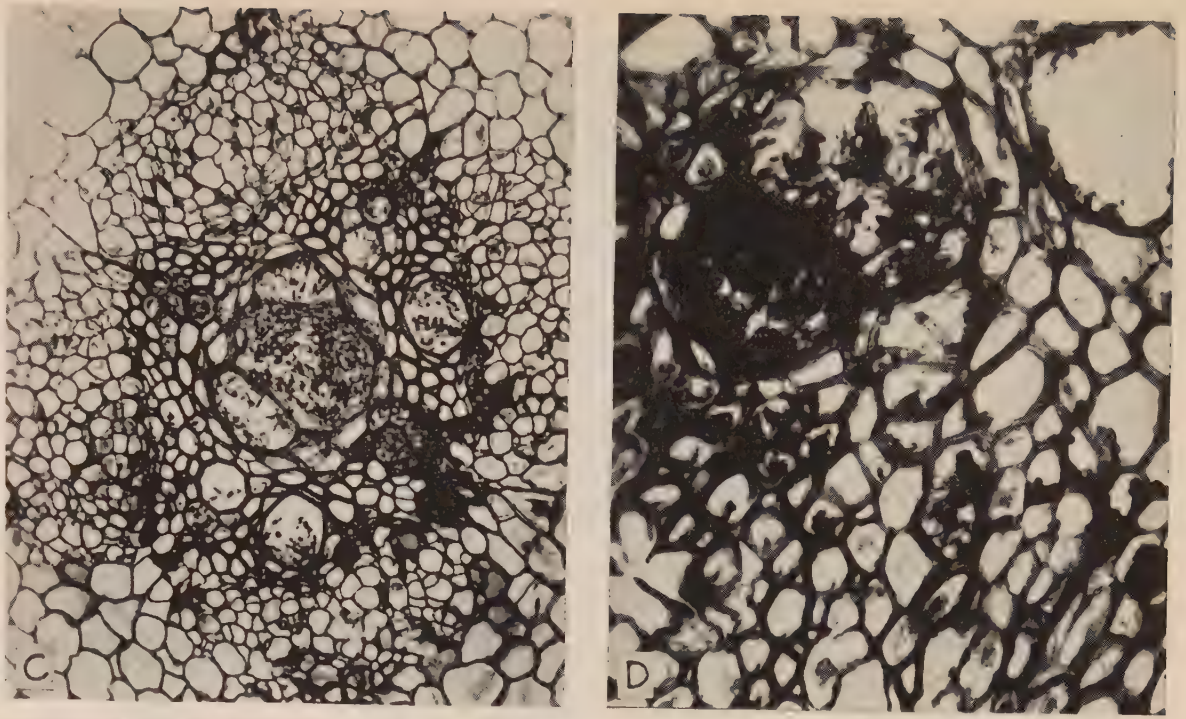

Fig. 4. A. Transverse section of a vascular bundle in the hypocotyl of healthy 40-day-old plant. X184. B. Vascular bundle in the hypocotyl of a wilted 40-day-old plant. Gum-like materials filling some tracheal vessels. Mycelium and small quantity of gum in others. Cells bordering xylem have a thickening in walls and protoplasm deeply stained. X368. C. Transverse section of a primary root, wilted 20-day-old seedling. Vessels heavily congested with mycelium and xylem parenchyma deeply stained with newly formed gum-like material. X184. D. Transverse section of a hypotocyl, 40-day-old wilted plant, showing gum-like material and mycelium in large tracheal vessel, small tracheids filled with gum-like materials and mycelium. Note thickening in middle lamella of some tracheids and xylem parenchyma cells. X368.

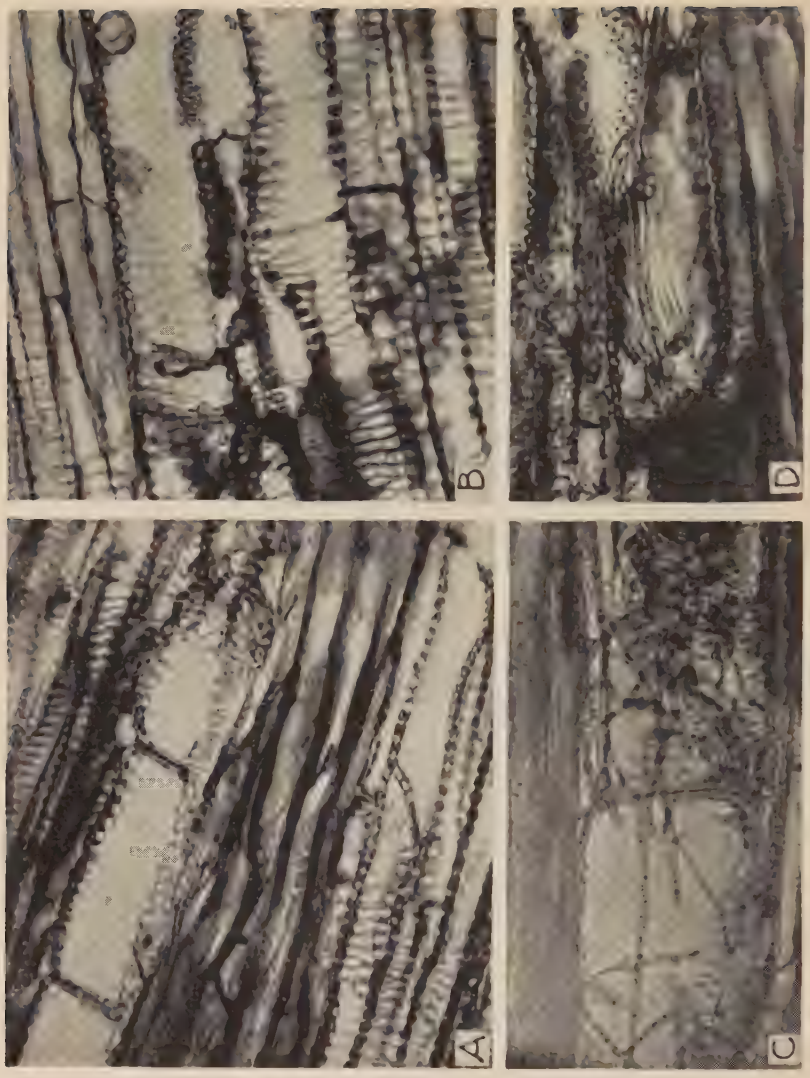
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FIG. 5. A. Longitudinal section. Hypocotyl of a 16-day-old healthy plant. Trachea and tracheids have a normal structure. X348. B. Longitudinal section of a primary root from infected 16-day-old Iowa Belle seedling. Gum-like material collecting over an end wall and tyloses extruding into lumen of a tracheal cell, secretions to ingress of the mycelium. X348. C. Longitudinal section of xylem in a hypocotyl of a 40-day-old plant. Mycelium massed in lumen of a tracheal cell and hyphae squeezing through pits of the end wall. X265. D. Longitudinal section, xylem in primary root, 20-day-old Iowa Belle seedling. Hyphs shrunken and sheathed with a gum-like material. Pits of the tracheal cell walls coated with the same material. X348. E. Longitudinal section of a hypocotyl near transition zone to primary root. Lumen of the tracheal cells completely congested with mycelium. Wilted 25-day-old seedling. X348. F. Longitudinal section of a hypocotyl of a 60-day-old wilted plant. Gum-like material coating the side walls, and walls and tyloses in the tracheal cells. X348.

water relationship is involved. Linford (33) has proposed that wilting of young pea plants infected with *Fusarium orthoceras* var. *pisi* Linford may be due to the loss of the normal powers of water retention by the leaf protoplasts rather than a diminished water supply through the vascular tissues. Smith (62), in his study of *Fusarium niveum,* attributed wilting of the watermelon plant to the lack of transpiration water brought about by partial or complete clogging of the water ducts by the pathogene. From the present study of watermelon seedlings infected with *Fusarium niveum* there is sufficient evidence to consider mycelial congestion in the root xylem a serious obstacle to the adequate movement of water and a potential cause for wilting.

SYMPTOMS ON STUNTED AND OLDER PLANTS

A more complex pathological relationship exists between the host and pathogene in stunted and older plants than in seedlings. Stunted plants exhibit a chronic diseased condition. Older plants which appear healthy but suddenly wilt suggest that an acute situation has developed. The internal structure of these plants indicates, however, that a pathological disturbance has been present in the xylem vessels for a long period.

EXTERNAL SYMPTOMS

Plants with the first and second true leaves are frequently stunted. Such plants at times develop straggling runners with short internodes, bloom profusely at the terminal nodes and occasionally produce an abortive melon before wilting. Young and old plants of this kind have greatly reduced root systems. Frequently the lower parts of the primary roots are completely

Fig. 6. Recovery of wilted seedlings in the moist chamber. A. Plant No. 1 in the initial stage of wilting, previous to being placed in the moist chamber. B. Three hours later. Plant No. 1 recovered.

disintegrated and a bushy growth of lateral roots develops near the crown.

Healthy appearing old plants which wilt abruptly in the field have well developed root systems. Root cankers such as described by Porter (47) may be present. Completely disintegrated lateral roots, diameter about 0.25 cm., appear occasionally along the side of the large primary root. Lesions form and extend into the primary root at the rupture made by the lateral root. This condition may be found at several points on the primary root and the roots branching from it. Older plants are subjected to attacks by the pathogene and infection involves a cumulative process. It was not determined whether other soil organisms might hinder or aid *Fusarium niveum* in gaining entrance through the lesions. Fawcett (16) has discussed such a relationship between pathogenes and secondary organisms in other plant diseases. Porter (47) secured a higher percentage of wilt when he used a mixture of manure and soil infested with *Fusarium niveum*. He considered that the additional organic matter aided the wilt pathogene by increasing its food supply.

INTERNAL SYMPTOMS

XYLEM

Invariably the xylem vessels are partially or completely browned in older watermelon plants when infected with *Fusarium niveum*. This discoloration appears more pronounced inside the stems near to the crown and in the primary root. Smith (62) pictured the xylem vessels filled with mycelium in his diagram of a cross section from a mature stem of a wilted watermelon plant. Gum-like materials and tyloses were not included. Substances other than mycelium were evidently recognized by Smith (62) who in his discussion of internal symptoms stated, "This browning of the xylem appears to be common to all plants attacked by soil Fusaria." He included watermelons among the hosts listed in support of his statement. Observations since those of Smith would indicate that many if not all organisms associated with vascular diseases induce a discoloration of the xylem. References may be consistently found where such occur (18) (20) (22) (25) (35) (56) (70).

Vessels of watermelon plants in which *Fusarium niveum* has been established for a considerable time are often gorged with gum-like materials and tyloses. These produce a brownish color in the tissues. This gum-like material and the unusual abundance of tyloses are intimately associated with the mycelium of the pathogene, and all three are involved in the pathological disturbances within the host. Gum-like materials are rarely present in vessels of healthy plants and tyloses are less abundant.

MYCELIUM

Mycelium appears more conspicuously in the younger xylem of older watermelon plants; however, relatively few of the ves-

sels are congested. The pathogene seems quite definitely limited to the trachea and tracheids and moves through the vessels in the same manner as in young plants. Walls of parenchyma and wood-fiber cells adjacent to an invaded vessel are darkly stained, their lumina being filled with materials which produce a brown granular effect (fig. 4, B, D).

Xylem vessels adjacent to those containing mycelium have gum-like accretions adhering to their inner walls (fig. 4, B). Occasionally hyphae of the pathogene are sheathed with the gum-like substance (fig. 5, D). These hyphae stain a lighter color and are shrunken. Such a situation suggests deteriorative action of the gum-like material. Apparently only the older hyphae were enveloped with the gum-like material since other hyphae in the same xylem cell were free from the gum-like sheath. During the study of infection in seedlings, it was observed that gum-like materials collected over the end walls and newly formed tyloses extruded through the pits in xylem cells of the primary root in advance of the hyphae when a lateral root was slowly invaded (fig. 5, B). This reaction of the xylem cells to the presence of the pathogene appears frequently in seedlings of resistant varieties.

GUM-LIKE MATERIALS

With due consideration to previous observations it would seem that in watermelon wilt the gum-like materials originate from the cells of the host rather than as a secretion or disintegration of the mycelium. Similar gum-like materials have been reported in the xylem of plants when attacked by other vascular fusaria (12) (13) (22) (35) (70). The presence of such materials in the xylem of the host is not specifically limited to fusarial diseases, but is frequently mentioned in studies of other plant pathogenes (18) (22) (25) (56) (72). Opinions vary in bacterial vascular diseases, particularly alfalfa wilt, as to whether the gums in vessels originate from metabolic residues of the bacteria or from injury to the host cells (25). Gum-like materials when associated with fungal plant diseases have been generally considered to be the products from cells of the host.

The nature of the influence which incites or stimulates gum-like formations and the chemistry of the reaction that accompanies it in plant cells have not been specifically determined. Different suggestions have been proposed, some of which involve autolytic cell-action (71), hydrolysis of the pectin in the cell wall (15), pectic enzymatic action (4) (23), and partial decarboxylation of pectin (39). Onslow (40) defines gum and mucilages as "abnormal products of the cell wall, the former being associated with injury which may bring about hydrations and oxidations in the wall, thereby greatly altering the chemical composition of the normal complexes, and producing other

complexes having the peculiar physical properties of this particular class."

Various "types of injuries" have been utilized to simulate the pathological symptoms that may occur in the host plant. Haskell (20) demonstrated that extracts of healthy potato tissues and various chemicals produced browning of the vascular elements in the potato plant. Overton (43) cites experiments in which gum formation occurred in the xylem of Cyperus after the epidermal and parenchymal tissues were injured by heat. In these instances no pathogene was involved, and only the diffusion of injured cell contents could be considered.

It would seem logical to associate the excessive formation of gum-like materials in the xylem of watermelon plants directly with the activities of the wilt pathogene. This change in the physiological processes within the host-cells may be due to one or many factors such as: (a) Elaboration of enzymes by the pathogene, (b) toxic action of metabolic products, (c) withdrawal of certain elements essential for the proper physiological balance of the cell sap, or (d) injury to the host cells which in turn affect other cells.

When cross sections are cut from the primary roots of resistant plants which have grown in soil heavily infested with *Fusarium niveum*, a ringed band of gum-like material and tyloses occludes the older xylem vessels at varying distances between the axis of the root and the outer active xylem (fig. 7, A). The location of the band appears to be contingent upon the age of the plant at the time infection occurred. Susceptible plants do not show this limitation of gum-like accretions. Resistant and susceptible plants are practically devoid of gum-like materials when grown in soil free of the wilt pathogene (fig. 7, B).

Seemingly healthy plants may harbor the wilt organism. This is especially true among wilt-resistant varieties of watermelons. Isolations from 500 resistant plants selected at random, which had matured a crop of melons in 1932 on soil heavily infested with *Fusarium niveum*, showed 40 percent of the root crowns to be harboring the pathogene. Subsequent tests proved the cultures from the isolations to be pathogenic to susceptible watermelon seedlings. Porter (47) also has isolated the organism from various parts of plants which appeared healthy. Resistant plants harboring the organism in the root crown seldom had it in the stems. Apparently the progress of the wilt pathogene was arrested or was limited to the root tissues. This situation did not occur in susceptible plants.

TYLOSES

Tyloses are a common anatomical feature in the xylem of cucurbits. Zimmerman (80), in a series of experiments with stems of *Peponium usambarense*, has made a critical study of how wound stimuli may incite tylosis formation. After evaluating the following theories relative to cause for tylosis develop-

Fig. 7. A. Transverse section of a mature primary root near the base of hypocotyl, showing a ringed band of gum-like material collected in the older xylem. Iowa Belle plant grown in soil heavily infested with *Fusarium* sirosum. X4.5. B. Transverse section of mature primary root. Iowa Belle plant, grown in soil free from infestation with *Fusarium* sirosum. X4.5.

ment: (a) Entrance of air into vessels, (b) rise in water pressure, (c) reduction in water pressure, (d) wounding, (e) change in tissue tension, (f) water loss in the vessel, and (g) interruption of water flow, he interpreted his experiments to indicate that the first and last theories were the most plausible. He concedes, however, that some other factor or factors might be involved.

Holroyd (23) speaks of "the abundant vascular tyloses" in the root of the watermelon, *Citrullus vulgaris*. His observations were confined to a histological study of mature plants. Neither the source of the plants nor their cultural history was mentioned. In observations made by the writer, tyloses are present chiefly in the older trachea near the central axis of the root. As the plant approaches maturity, tyloses appear in the larger trachea farther from the axis, but seldom are found in the more active, outer xylem. The outer active xylem layer, as determined by Gourley's (19) basic fuchsin method, was three to four cells thick. Plants growing in soil free of *Fusarium niveum* have fewer tyloses in the vessels of primary roots than do plants infected with the pathogene.

Lumina of vessels in plants infected with the wilt pathogene are frequently filled with tyloses in all stages of development (fig. 7, B). They may appear at the same time that gum-like accretions begin to collect over the end walls of vessels in young plants (fig. 5, B). In older plants the inner walls of the xylem vessels and the tyloses are often coated with gum-like materials (fig. 5, F).

Wardlaw (70) differentiates between healthy and diseased tyloses in banana plants infected with *Fusarium cubense*. He considers the death of the diseased tyloses to be caused by toxic substances. Autolysis of the tylose involved a disintegration of the nucleus and cytoplasm, followed by a browning of the matrix, which finally acquired the yellow-brown color of the gum which enveloped it. Tyloses in diseased watermelon plants present a similar appearance. Sleeth (59) recognizes an apparent pathological relationship between the pathogene and the formation of tyloses in watermelon plants infected with *Fusarium niveum*. He does not mention in his brief report the occurrence of gum-like materials. In the observations made by the writer, gum-like materials are intimately associated with tyloses in plants infected with the wilt pathogene (fig. 5, F). The two undoubtedly occur as a reaction of the host cells to the presence of the pathogene.

GREENHOUSE AND FIELD STUDIES

Since none of the known edible varieties of *Citrullus vulgaris* is immune to wilt, it has been necessary to search for resistant segregates within the species. The usual procedure, first employed by Orton (42), was to grow large plant populations in the field on soil heavily infested with *Fusarium niveum*. By this

method he developed and introduced the first wilt resistant variety, known as "Conqueror." This variety was a resistant segregate from a backcross of Eden, the edible susceptible parent, to the F_1 hybrid, Eden x Stock Citron.

Further development of new resistant varieties was begun by this station in 1925 when chance hybrids were selected from the Conqueror and controlled hybridization was initiated (42). This was followed by extensive varietal tests, selection, crossing, and inbreeding of resistant segregates, and to a limited extent by the hybridization of edible susceptible varieties with resistant inedible sorts. The results of this work and associated studies of the host and pathogene have been reported by Porter (45) (46) (47) (48) (49), Porter and Henderson (51), Porter and Melhus (52) (53), Melhus *et al.* (38), Layton and Wilson (29), Wilson (81), and Wilson and Layton (73) (74). During the course of these investigations it became evident that resistance could be measured more accurately if the pathological relationship between the host and pathogene were better understood. The present greenhouse and field studies were organized with two objectives in mind: (a) To facilitate the isolation and increase of more resistant selections, and (b) to provide a clearer understanding of the pathological problems involved.

GREENHOUSE INDEXING 1931 AND 1932

In developing wilt-resistant watermelon varieties, the practice has been to grow plants in fields known to be heavily infested with *Fusarium niveum*. Seed from flowers fertilized by controlled pollination was saved from surviving plants. Each succeeding generation increased the total to be tested for resistance. This soon resulted in unwieldy numbers. A method was sought whereby the resistance or susceptibility of seedlings might be ascertained in the greenhouse during the winter season, thereby reducing the acreage needed for such a test in field plots.

MATERIALS AND METHODS

Porter and Melhus (53) accomplished this result by planting random samples of individual seed lots in 4-inch clay pots filled with steamed soil which had been dosed with inoculum of the wilt organism. In this study, however, their method has been modified. Five seeds of a wilt-resistant melon and an equal number from a bulk lot of a susceptible variety, Kleckley Sweet, were planted in opposite halves of a 4-inch clay pot. Each individual selection was planted in duplicate in the 1931 indexing studies, and in triplicate in the 1932 studies. Steamed soil, consisting of 1 part compost mixed with 2 parts fine sand, was used. A leveled tablespoon of wilt-infested soil was stirred into each pot at the one-third level. Soil inoculum for greenhouse studies was prepared from steamed soil heavily dosed with a mixture of *Fusarium niveum* cultures in which susceptible plants were grown

until all of them had wilted. Eight cultures collected in Iowa and six from other states were chosen from the stock cultures of *Fusarium niveum* because of their known pathogenicity. Sleeth (60) has reported a variability in the pathogenicity of cultures. His tests verify observations made by the writer relative to the respective high and low pathogenicity of two cultures supplied from this laboratory. Cultures isolated directly from the infected plant have proved more pathogenic than those kept in stock.

Soil temperatures ranged from 20° to 25° C. during the indexing studies. Seedlings were counted at emergence, 6 to 9 days after planting. Wilting usually appeared 3 to 4 days later. The wilted plants in resistant selections and checks were counted separately and removed at 1- to 4-day intervals for periods of 18 to 22 days. The total percentages of wilted seedlings for each selection and its check were determined by dividing the initial stand into the total wilted at any given reading. Percentage of average daily wilt between readings was computed by dividing the number of surviving seedlings at the preceding reading into the average number which wilted each day during the period. Percentages were computed by the same method in the data from field experiments. Individual selections having a low percentage of wilted seedlings when indexed were taken to the field plots.

INDEXING IOWA KING SELECTIONS

Thirty-nine F_5 Iowa King selections were indexed during the winter in 1931 and 106 F_6 selections were indexed in 1932. A summary of the results from these studies is presented in table 1. At the end of 29 days 95 percent of the Iowa King selections and 99 percent of the checks in Series I had wilted. Because of the high mortality in the first series, a second was prepared. Instead of using a soil inoculum infested with a mixture of *Fusarium niveum* cultures, a single culture was used. This culture had been isolated from a wilted plant taken in the field plot where the Iowa King selections were produced the previous year. Eleven selections with the lowest percentage of wilt in Series I were chosen for indexing in Series II. Very high percentages of wilt occurred again with no one of the selections outstanding in resistance.

In 1932 one series of 106 F_6 seed melons was indexed. Readings were discontinued 18 days after planting when 65 percent of the seedlings in the test selections and 64 percent of those in the checks had wilted. Apparently seedlings of the F_6 Iowa King selections were unable to withstand the heavy infection induced by the dosage of *Fusarium niveum* used in the experiment. It has been shown (47) (53) that seedlings of highly resistant sorts such as inedible citrons succumb to wilt when their root systems are restricted to a small area and are exposed to repeated infection from a heavy dosage of *Fusarium niveum*.

INDEXING PRIDE OF MUSCATINE SELECTIONS

Three hundred F_4 inbred selections of the Pride of Muscatine variety were separated into two series for indexing in 1931. Series I was planted 3 days in advance of Series II. The reaction of the Pride of Muscatine selections was of particular interest since the selections were the F_4 inbred progeny of a resistant segregate (53) isolated from the susceptible variety, Kleckley Sweet. The percentage of wilt and percentage average daily wilt, as shown in fig. 8, were consistently less throughout the indexing test in Pride of Muscatine seedlings than in the Kleckley Sweet check. The greatest difference appeared during the first 24 days after planting when the percentage of wilted seedlings in the check was approximately twice that of Pride of Muscatine.

INDEXING IOWA BELLE SELECTIONS

Seedlings of Iowa Belle selections proved to be more resistant in the indexing tests than seedlings of Iowa King and Pride of Muscatine. A greater resistance to the wilt organism was equally apparent in later field trials. For this reason detailed studies were made of F_3 and F_4 Iowa Belle selections in the greenhouse during the winter months of 1931 and 1932, respectively. Exact genetical ratios could not be computed because only a part of the progeny was inbred and harvested from any one parental line when grown in the field. In order that the greenhouse indexing tests might be more readily evaluated, selections of parental lines were classified into four arbitrary groups according to whether 0 to 24, 25 to 49, 50 to 74, or 75 to 100 percent of the seedlings wilted. Selections placed in the first two groups were considered to be highly resistant, while those in the latter two groups were not. Resistance as expressed by individual selections in the seedling stage was based upon a low percentage of wilted plants.

One hundred and twenty-six F_3 Iowa Belle selections, the progeny of 30 F_2 parental lines, were arranged into the above four arbitrary groups (table 2) according to the percentage of seedlings wilting in the greenhouse. When classified in this manner 7.2 percent fitted into group I, 27 percent in group II, 30.1 percent in group III, and 35.7 percent in group IV. Sixteen selections from groups I and II and nine selections from groups III and IV were chosen for further study and increase in field plots during 1931.

Two hundred and fifteen F_4 selections, progeny of the above 1931 selections, were indexed in the greenhouse in 1932. These have been classified into four arbitrary groups in table 2 according to the percentage of wilted seedlings in each selection. Group I, which contained the most resistant selections, was 3.9 percent larger than in 1931. Group II, next in resistance, increased 21.4

percent, and group III gained 5.7 percent, while group IV, the least resistant, decreased 31.0 percent. This shift to higher percentages in resistance of F_4 Iowa Belle selections was apparently due to the elimination of the less resistant F_3 selections by greenhouse indexing. When planted in field plots heavily infested

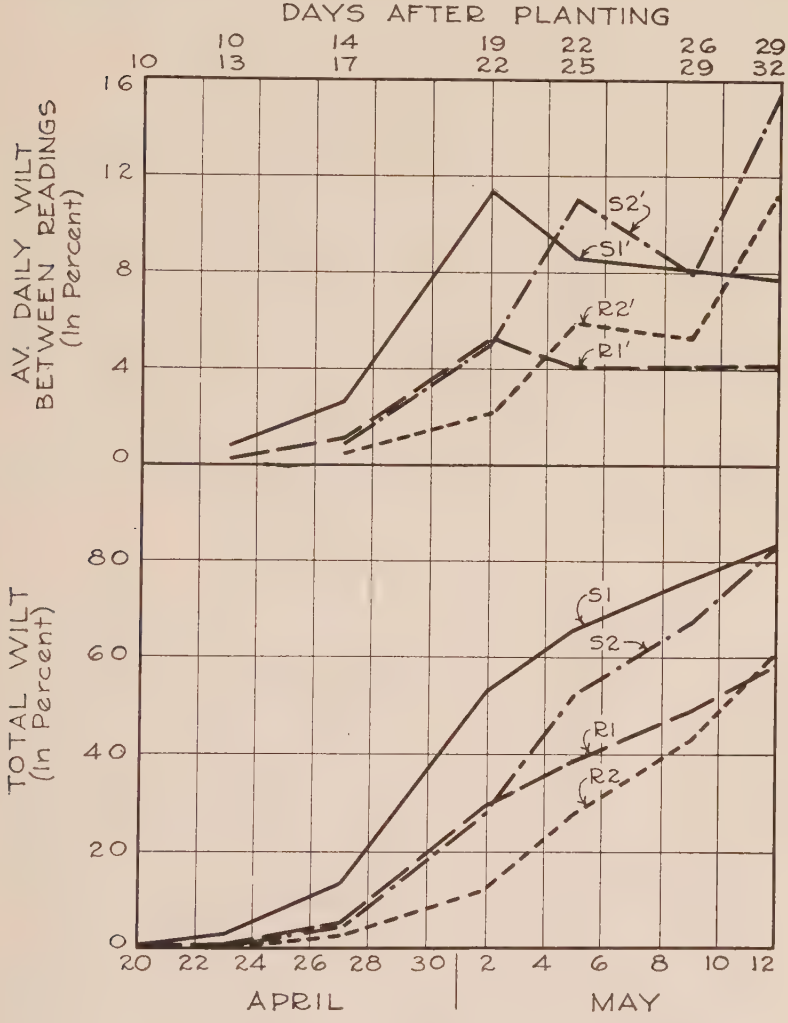

Fig. 8. Percentage of average daily wilt and total wilt in seedlings of Pride of Muscatine selections and susceptible checks at different periods during greenhouse indexing test in Series I and Series II, 1931. Checks identified as (S1) total wilt, (S1′) average daily wilt in Series I; and (S2) total wilt, (S2′) average daily wilt in Series II. Pride of Muscatine selections identified as (R1) total wilt, (R1′) average daily wilt in Series I; and (R2) total wilt, (R2′) average daily wilt in Series II.

with the wilt pathogene, these selections were subjected to further segregation for resistance since only the surviving plants were inbred and harvested.

Results in greenhouse indexes for resistance within selections show thus far that a technique applicable to one resistant variety may not be equally usable with another. Iowa King selections, when subjected to the same treatment as Iowa Belle selections in greenhouse indexing tests, did not exhibit an equal degree of relative resistance.

FIELD STUDIES

Field tests in 1929 and 1930 were planned to develop a higher percentage of resistance in selections obtained from earlier field experimentation while in 1931 and 1932 the tests were based on those selections which showed some resistance in the greenhouse indexing tests. Practically all breeding operations, such as selfing, inter-selection crossing, hybridizing and backcrossing, were performed with plants in the field. When sufficient numbers of highly resistant inbred selections with desirable, homozygous, morphological characters were obtained more of their progeny were selected.

A count of the plants killed by *Fusarium niveum* in each selection was taken at intervals throughout each growing season. These data have been assembled with the daily air and soil temperatures and the amount of precipitation.

MATERIALS AND METHODS

The data in the field tests deal with the entire plant population of the varieties Iowa King, Pride of Muscatine and Iowa Belle. Individual selections within varieties, however, were treated on a separate statistical basis and their further increase was decided according to their percentage of resistance, vigor, productiveness, size and quality of fruit, earliness and uniformity in maturity. Susceptible checks of the variety, Kleckley Sweet, were planted each season. The checks were planted every sixth melon hill in the row in the 1930, 1931 and 1932 studies. Hills between the checks were planted to resistant selections. Each selection was replicated three to eight times, depending on the supply of seed.

Percentages of average daily wilt and of total wilt were computed by the method used in the greenhouse indexing studies. Each season the field studies were separated into two periods. During the first period the total percentage of wilted plants was computed from the number of seedlings emerged at the time of the initial stand count. At the beginning of the second period in early July the resistant plants were thinned to two per hill and a new stand count was taken. The percentage of wilt in the second period was then based upon the revised stand count. At the beginning of the second period all plants re-

TABLE 1. RESULTS OF INDEXING IOWA KING SELECTIONS. GREENHOUSE STUDIES, 1931 AND 1932.

Series number	Number selections	Initial stand	Percentage of wilt—Days after planting														
			9	10	11	12	13	14	16	18	19	21	24	25	28	29	30
1931 Series I (duplicate)																	
Iowa King (F_5)	39	347	0.0		5.8	71.2			89.3			92.8	94.5			95.4	
Kleckley Sweet (check)		364	0.8		9.0	64.0			91.2			95.3	99.5			99.5	
*1931 Series II																	
Iowa King (F_5)	11	104		1.7			4.8				52.9	68.3		75.0	86.5		92.3
Kleckley Sweet (check)		89		0.0			0.8				34.9	51.7		64.0	83.1		94.4
1932 Series (triplicate)																	
Iowa King (F_5)	106	1482	1.4		7.2	16.5		24.3		65.3							
Kleckley Sweet (check)		1405	1.7		5.8	16.2		25.6		64.3							

*Series II in 1931 represented seed melons included in Series I of same year.

TABLE 2. PERCENTAGE OF THE F_3 AND F_4 IOWA BELLE SELECTIONS IN EACH OF FOUR GROUPS WHEN SORTED ACCORDING TO PERCENTAGE WILTED. GREENHOUSE INDEX, 1931 AND 1932.

Year and selections	Number of selections	Percentage of total seedlings wilted				Percentage wilted in checks
		I 0–24%	II 25–49%	III 50–74%	IV 75–100%	
1931 F_3 selections	126	7.2	27.0	30.1	35.7	95.0
1932 F_4 selections	215	11.1	48.4	35.8	4.7	92.0
Difference between 1932 and 1931 percentages		+3.9	+21.4	+5.7	−31.0	−3.0

maining in the checks were removed in order to protect the purity of the resistant plants, which were always used to produce seed.

Air and soil temperatures were recorded automatically by a thermograph. The soil temperature was taken at 6 inches below the surface, while daily precipitation was measured over 24-hour periods at 7 a. m. Temperature and precipitation records were kept from the time the field plots were planted until the close of the growing season. That part of the meteorological data included in the first half of each growing season has been given specific consideration in the present study.

FOUR-YEAR FIELD TESTS OF THE IOWA KING VARIETY

Inbred selections of the wilt-resistant variety, Iowa King, were grown 4 successive years, 1929 to 1932, in Field No. 1. The soil in this field was moderately infested with *Fusarium niveum*, which became more abundantly distributed each year that melons were grown. This was shown by the check seedlings, in which the numbers wilting increased from 34 percent in 1929 to 81 percent in 1932 (table 3). With 1931 excepted, the percentage of wilted seedlings among the resistant Iowa King selections increased each year. There was, however, a lower percentage of wilt in the Iowa King seedlings than in those of the check, the difference being 15.7 percent in 1929, 23 percent in 1930, 27.2 percent in 1931, and 35.8 percent less in 1932.

Very few of the resistant plants wilted during the latter half of each season. This was particularly true in 1930, 1931 and 1932. The high percentage of wilt in the Iowa King plants during the latter half of 1929 may have been due, in part, to a greater number of susceptible segregates in the F_3 progeny having escaped early infection in the seedling stage. It would seem logical for a later infection to shift the mortality into the latter half of the growing season. Such a situation frequently occurs when susceptible commercial varieties are grown in soils

TABLE 3. DATA FROM 4-YEAR FIELD TESTS OF WILT RESISTANT VARIETY, IOWA KING. FIELD NO. 1. 1929, 1930, 1931, 1932.

Year and selections	Planting date	Number plots	First half of growing season															Last half of growing season			Years in melons
			Initial stand	Percentage of plants wilted—Days after planting														Revised stand	Days observed	Percentage wilted	
				12	16	18	23	24	27	28	30	31	32	36	37	42	44	50				
1929	May 22																					
F_3 Inbreds		12	475						9.7					16.4				18.3	390	68	25.9	1
Check		4	138						18.1					22.5				34.0	—	—	—	
1930	May 20																					
F_4 Inbreds		50	1468				3.4					13.5					21.5		678	94	3.1	2
Check		55	461				8.9					33.4					44.5		—	—	—	
1931	May 19																					
F_5 Inbreds		32	2352				4.0						12.6			14.5			597	88	4.0	3
Check		40	487				10.7						30.4			41.7			—	—	—	
1932	May 19																					
F_6 Inbreds		60	2169	2.4	4.2	12.8		30.2		34.1		40.2			42.9	45.2			452	91	14.8	4
Check		75	657	2.0	5.0	12.8		35.4		43.5		61.0			75.6	81.0			—	—	—	

TABLE 4. DATA FROM 3-YEAR'S FIELD TESTS OF WILT RESISTANT VARIETY, PRIDE OF MUSCATINE. FIELD NO. 2. 1929, 1930, 1931.

Year and selections	Planting date	Number plots	Initial stand	First half of growing season													Last half of growing season			Years in melons
				Percentage of plants wilted—Days after planting													Revised stand	Days observed	Percentage wilted	
				8	16	19	23	24	28	30	31	35	37	38	42	47				
1929	May 18																			
F_2 Inbreds	June 4*	95	2062	4.8	9.1		20.1		26.7				29.5				959	68	32.7	4
Check		21	1636	8.4	24.2		49.0		58.9				71.7				—	—	—	
1930	May 21																			
F_3 Inbreds		137	4960			13.5				36.4				42.8		44.0	2086	96	36.0	5
Check		28	800			27.1				53.5				72.9		86.4	—	—	—	
1931	May 18																			
F_4 Inbreds		140	6575		9.7			25.1	30.2		33.3	35.8			36.6		2119	85	43.5	6
Check		140	1109		4.1			31.7	41.4		50.0	57.5			67.1		—	—	—	

*Date when missing hills were replanted.

infested with *Fusarium niveum*. In the present case, watermelons had not been planted in Field No. 1 during the preceding 9 years. The increase in percentage of wilted plants among the resistant selections each consecutive year might, at first thought, be attributed to a loss in resistance; however, similar increases occurred in the susceptible checks. Successive cropping with watermelons apparently had increased the degree of wilt infestation in the soil.

Fifteen individual F_6 inbred selections were used in the 1932 field study. These selections represented the progeny of five segregates of the F_3 selection used in 1929. It would seem that resistance should have been fairly constant, since only surviving inbred progeny had been selected during six generations. Even with inbreeding and selection, resistance to wilt appeared to be a relative factor. In the first half of the 1932 growing season 45.2 percent of the Iowa King seedlings died of *Fusarium niveum*. The mortality was much lower in the last half of the season, only 14.8 percent of the older plants wilting. After repeated crops of watermelons, the soil in Field No. 1 had become heavily infested with *Fusarium niveum*. As a result, plants were subjected to frequent early infection and the percentage wilting increased each year. The total percentage wilting in the susceptible checks, however, remained nearly twice that in the resistant selections.

THREE-YEAR TESTS OF THE VARIETY, PRIDE OF MUSCATINE

The resistance of F_2, F_3 and F_4 inbred selections of Pride of Muscatine to the wilt pathogene was studied in Field No. 2 in 1929, 1930 and 1931. This field had been utilized in similar studies of other varieties for 3 consecutive years prior to the beginning of the present experiments. It was impossible to mature susceptible varieties of watermelons in this field, because of wilt; hence, resistant sorts were subjected to a particularly severe test.

In the first year, 1929, 71.7 percent of the seedlings in the checks wilted within 37 days after being planted (table 4). During the same period 29.5 percent of the Pride of Muscatine seedlings died. The mortality in the first half of 1930 was still higher, 86.4 percent in the checks and 44.0 percent in the resistant selections. There was a reduction in the percentage of wilt the first half of 1931. It will be recalled that the same situation occurred in Field No. 1 (table 6). This situation will be given further consideration in a later chapter dealing with the meteorological data.

THREE-YEAR FIELD TESTS OF THE VARIETY, IOWA BELLE

It will be recalled that resistance to the wilt organism among inbred segregates of the Iowa Belle had been consistently out-

TABLE 5. DATA FROM 3-YEAR FIELD TESTS OF WILT RESISTANT VARIETY IOWA BELLE. FIELD NO. 3, 1930, 1931, AND FIELD NO. 2, 1932.

Year and selection	Planting date	Number plots	Initial stand	First half of season: Percentage of plants wilted—Days after planting														Revised stand	Days observed	Percentage wilt	Years in melons
				12	14	16	18	21	22	23	25	27	32	33	35	37	43				
1930	May 15																				
F_2 Inbreds		18	592									29.1			43.9		48.3	128	94	8.6	3
Check		6	206									26.2			42.7		62.2	68		44.1	
1931	May 18																				
F_3 Inbreds		267	3925							14.4	19.2		20.2				20.2	1608	85	0.2	4
Check		312	3075							6.5	25.5		39.3				48.6	—	—	—	
*1932 (Series I)	May 18																				
F_4 Inbreds		192	3362			4.5	12.5	22.4				35.2		38.8	39.4	39.8	41.0	1840	91	0.7	7
Check		216	1265			5.0	16.7	29.0				52.8		72.2	77.5	79.7	83.5	—	—	—	
*1932 (Series II)	June 8																				
F_4 Inbreds		170	1963	9.2	17.8	25.0			30.7									—	—	—	7
Check		106	691	11.1	27.4	48.0			68.2									—	—	—	

*Data represents observations on Field No. 2 planted with F_4 Iowa Belle Inbreds in 1932.

standing in the greenhouse indexing studies. This variety was grown in 1930 and 1931 in Field No. 3, which had a history similar to Field No. 2, except that parts of the field had been cropped with watermelons one year less. During the first half of the season in 1930, 48.3 percent of the F_2 Iowa Belle selections wilted (table 5). Apparently most of the susceptible segregates were eliminated in the early season mortality, since in the latter half of the season only 8.6 percent of the remaining stand died due to *Fusarium niveum*. There was more than 50 percent less wilt in seedlings of the resistant selections than in those of the check in 1931. Neither the resistant seedling nor the checks, however, wilted in as great numbers as in 1930. This peculiarity appeared also in data obtained from 1931 studies of Iowa King in Field No. 1 (table 3), and Pride of Muscatine in Field No. 2 (table 4). Little wilting occurred in the Iowa Belle plants after the middle of the growing season. Only 0.2 percent wilted in 1931 and 0.7 percent in 1932. The remarkable resistance of this variety in 1932 was particularly interesting, because it was grown in Field No. 2 (table 5), where 43.5 percent of the Pride of Muscatine plants wilted in the last half of 1931. Only 0.7 percent of the Iowa Belle plants died of wilt in the last half of 1932.

FOUR-YEAR FIELD STUDY OF METEOROLOGICAL DATA INCIDENT TO WILT STUDIES IN FIELD TESTS

In the present field studies the daily maximum air temperature, maximum and minimum soil temperature and precipitation have been assembled with the percentage total wilt and percentage average daily wilt observed in resistant and susceptible watermelon seedlings at periods throughout the first half of each growing season in 1929, 1930, 1931 and 1932. These observations were taken in conjunction with the experimental studies conducted in Field No. 2 (tables 4 and 5). Total percentages of wilt have been designated "R" for resistant selections and "S" for susceptible checks. Percentages of daily wilt are identified as "R′" for resistant selections and "S′" for susceptible checks. The meteorological data and the trend of "R" compared with "S" and "R′" compared with "S′" have been plotted for each year (figs. 9, 10, 11, 12). These depict graphically the relationship involved.

METEOROLOGICAL DATA INCIDENT TO WATERMELON WILT STUDIES IN 1929

Figure 9 shows that the highest percentage of average daily wilt in susceptible and resistant seedlings occurred during the first 16 to 23 days after planting. After this time the numbers wilting declined rapidly in the resistant seedlings and the average daily mortality in the susceptible seedlings never reached the previous high point. Frequent precipitation occurred in

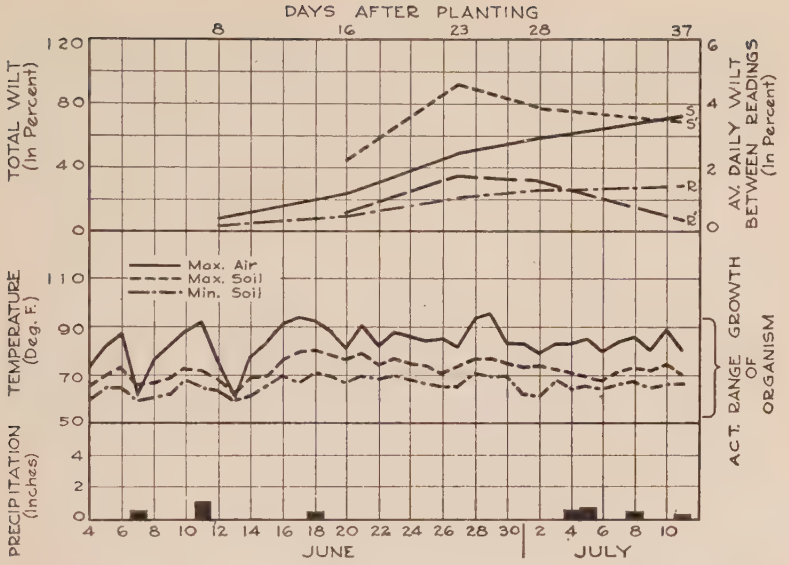

Fig. 9. Temperatures, rainfall and percentage wilt in Field No. 2, 1929. (S) total wilt and (S') average daily wilt in checks. (R) total wilt and (R') average daily wilt in resistant selections.

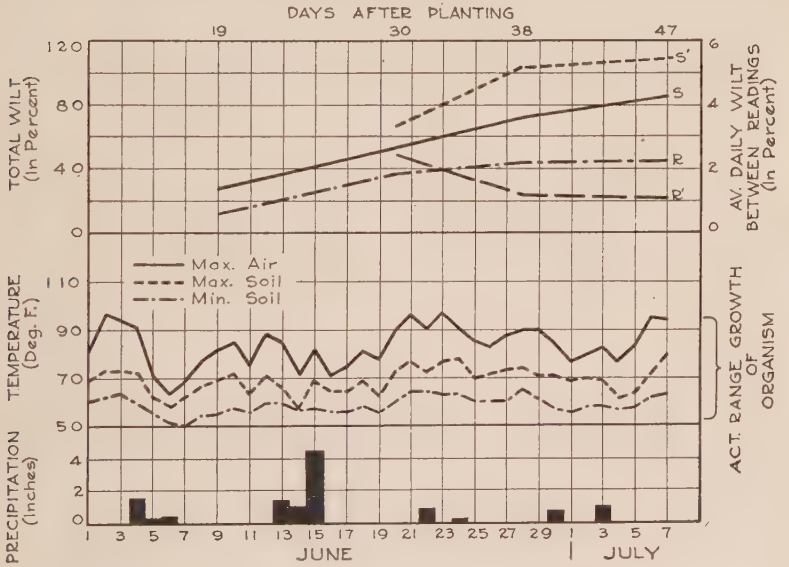

Fig. 10. Temperatures, rainfall and percentage wilt in Field No. 2, 1930. (S) total wilt, (S') average daily wilt in checks. (R) total wilt, (R') average daily wilt in resistant selections.

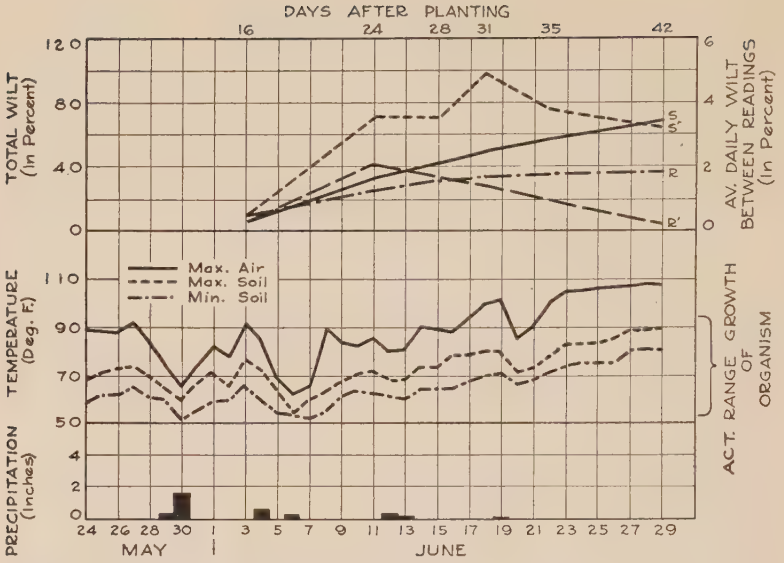

Fig. 11. Temperatures, rainfall and percentage wilt in Field No. 2, 1931. (S) total wilt, (S′) average daily wilt in checks. (R) total wilt, (R′) average daily wilt in resistant selections.

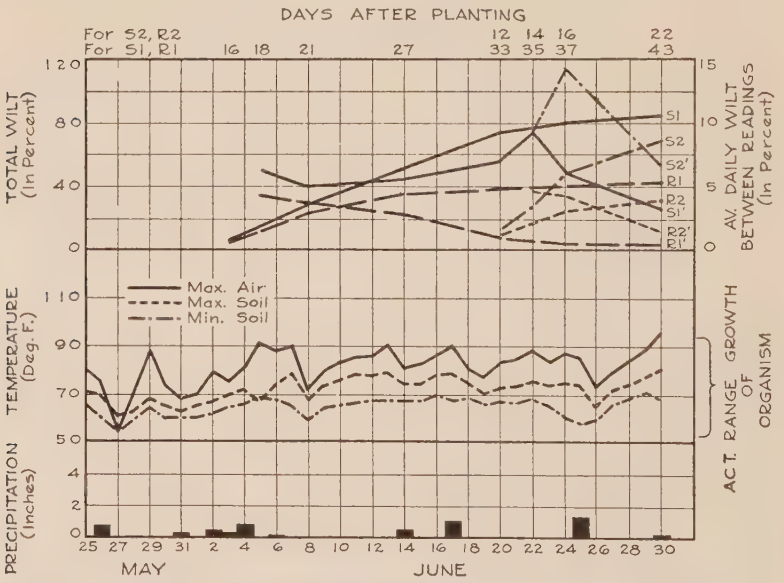

Fig. 12. Temperatures, rainfall and percentage wilt in Field No. 2, 1932. Series I (S1) total wilt, (S1′) average daily wilt in checks. (R1) total wilt, (R1′) average daily wilt in resistant selections. Series II (S2) total wilt, (S2′) average daily wilt in checks. (R2) total wilt, (R2′) average daily wilt in resistant selections.

the last period of the study and the air temperatures declined. Soil temperatures were within the range for active growth of the pathogene throughout the entire first half of the season. There were no extreme changes in temperature after the first 8 days following the planting date. Wilting among resistant seedlings declined 28 days after planting irrespective of the environmental changes.

METEOROLOGICAL DATA INCIDENT TO WATERMELON WILT STUDIES IN 1930

The periods between the three readings in this study were so widely separated that any variations in the percentages of average daily wilt within periods were obscured (fig. 10). Note that the percentage of average daily wilt in the resistant seedlings followed a downward trend, while in the susceptible seedlings the percentage consistently increased. Wilting in the resistant selections increased very little after 35 days from planting irrespective of environmental factors, while in susceptible plants there was a progressive increase.

METEOROLOGICAL DATA INCIDENT TO WATERMELON WILT STUDIES IN 1931

It will be recalled that the percentage of wilt was consistently lower in 1931 than in 1930 among resistant and susceptible plants studied in Field No. 1 (table 3) ; Field,No. 2 (table 4), and Field No. 3 (table 5). A discussion of this departure from the expected response was reserved, at the time, until the meteorological data could be considered. These data have been included in fig. 11. Scarcely any precipitations occurred the last half of the 35 days included in the observation. Air temperatures were unusually high, above 90° F. except on 2 days. Soil temperatures were similarly above normal. Such a situation should presumably afford an ideal environment for the expression of wilt. Porter (50) has observed that greater numbers of watermelon plants wilted when the air temperatures rose abruptly in the field following cool nights. There were only 4 nights with air temperatures below 70° F. in the last 20 days of the present study, and in those instances the temperature was above 60° F. After the first 7 days in June the remainder of the month was characterized by gradually increasing temperatures with scarcely any precipitation. Watermelon plants made an uninterrupted growth. Naturally the question arises as to what if any influence these factors of environment may have had upon the expression of wilt.

There was a gradual increase in the percentage of average daily wilt in the susceptible seedlings until 31 days after planting. The increase seemed to be correlated with air and soil temperatures up to this point, after which the rate of wilt de-

clined while air and soil temperatures rose to higher levels than during any of the previous periods. The percentage of average daily wilt in resistant seedlings began to decline 24 days after planting. A similar decrease in wilting occurred in the susceptible controls and resistant selections grown in three separate fields (tables 3, 4 and 5). Apparently the absence of fluctuating environmental factors may have stabilized the host and made it more difficult of attack by the pathogene. Also the very high temperatures may have retarded the activity of the pathogene.

METEOROLOGICAL DATA INCIDENT TO WATERMELON WILT STUDIES IN 1932

Two series of plantings 21 days apart were used in 1932 to determine the relative percentage of wilting in Iowa Belle plants of different ages when grown under similar environmental conditions. Series 1, planted May 18, has been designated "S1," susceptible, and "R1," resistant, while Series II, planted 21 days later, has been marked "S2," susceptible, and "R2," resistant, in fig. 12.

A high mortality from wilt appeared in the plants of Series I, 18 days after planting. Following 3 days of intermittent precipitation, there was a sudden rise of temperature on June 5, accompanied by a high percentage average daily wilt in Series I. This rise in temperature was followed by a sharp decline, on June 8, with a subsequent reduction in numbers of wilted plants. From this point the percentage average daily wilt in susceptible plants began to rise slowly until 35 days after the date of planting when a second decline in numbers wilting occurred. The percentage average daily wilt in the resistant Iowa Belle seedlings followed an entirely different trend. The numbers wilting declined continuously from the first reading, 16 days after planting. Few plants wilted after 35 days, the total wilt being less than 1 percent during the last half of the season (table 5).

Susceptible seedlings in Series II, planted June 8, began to wilt rapidly on June 22, the highest percentage average daily wilt being reached 16 days after planting. During the same period the percentage average daily wilt decreased in the susceptible check plants of Series I and in the resistant plants of Series II.

In all 4 years of the field tests there was a tendency toward a gradual increase in the average daily wilt, which followed closely the age of the seedlings and reached the highest point in susceptible checks at about 35 days after planting. The maxima were lower in the resistant seedlings and occurred at from 16 to 24 days after planting, following which there was a rapid decline. Environmental factors which were studied in

these field experiments temporarily interrupted or accelerated this trend and altered the points of the maxima, but did not change the general course of the host's reaction to the pathogene.

DISCUSSION

In the present study of watermelon plants attacked by *Fusarium niveum*, it has been found that root tips and ruptures formed by newly emerged lateral roots are the principal points of infection. Invasion was not necessarily limited to the meristematic tissues at the tip as designated by Yoshii (75). Epidermal cells at the side of the root 2 mm. to 6 mm. back of the tip in the zone of elongation and maturation were readily penetrated by hyphae of *Fusarium niveum*, which continued intracellularly through the cortical tissues to the pericycle. Further advance was effected by a massing of the hyphae and the subsequent penetration of the endodermis, following which the xylem vessels were rapidly invaded (fig. 2, C).

Lesions, when present at the ruptures formed by recently developed lateral roots, were the result of infection by *Fusarium niveum* at these points. This was substantiated by the isolation of pure cultures of the organism from such root tissues. The progress of the invading mycelium was also followed in paraffin sections of the root. Mycelium was noted spreading through the cortical tissues and extending inward along the ruptures formed by the lateral root. Endodermal cells of the lateral and parent root were destroyed after the mycelium had massed at the pericycle (fig. 1, C). Conant (9) has explained a similar breaking down of the endodermis in seedling tobacco roots, when the mycelium of *Thielavia basicola* Zoph. became massed, as the result of suberized and lignified cell walls being changed to pectin-like materials.

Evidence presented suggests that watermelon plants, during their entire growth period, were exposed to attack by *Fusarium niveum* through the continuous development of lateral roots. Early infection of this nature occurred among seedlings in the field when grown in soil heavily infested with the wilt pathogene or in the greenhouse when confined to pots with a similar soil infestation. Xylem vessels in primary roots of wilted seedlings, 15 to 30 days old, were invariably congested with mycelium at a point about 2 cm. below the base of the hypocotyl. In seedlings of this age the root xylem consists chiefly of several relatively large tracheae in the center of the tetrad and smaller vessels in the "rays" (fig. 4, C). Apparently when these vessels became congested with mycelium, insufficient water was conducted to compensate for that lost through transpiration, and the plants wilted. This explanation of seedling wilt was

sustained by placing seedlings showing the first symptoms of wilting in a moist chamber where they recovered their turgescence (fig. 6, A, B). No further symptoms of wilting appeared while the plants were kept in an atmosphere near the saturation point where little or no transpiration took place. Wilting, however, is believed to be not wholly the result of mycelial congestion in the vessels, since gum formation and tyloses begin to appear in the xylem of seedlings about 30 days old.

Gum-like materials and tyloses intermixed with mycelium occurred in great abundance in the xylem vessels of older plants, 40 to 90 days old, which had suddenly wilted while in apparently unimpeded growth. Although only a part of the xylem vessels of the stem were affected, those in the primary root axis were filled, giving to the structure a brown appearance. That gum-like materials and tyloses in the xylem vessels might seriously interfere with water conditions in the vessels was demonstrated by tracing the movement of a basic fuchsin solution as used by Gourley (19). When roots of healthy watermelon plants were submerged and cut in the basic fuchsin solution, the first three to four layers of secondary xylem cells nearest the cambium of the primary root were stained a deep red in a few hours. Diseased plants which had not wilted were treated in the same manner with the result that many of the secondary xylem vessels remained unstained. Upon examining free hand sections of the root the unstained vessels were found to be filled with gum-like material and tyloses.

The significance of any interference with transportation of water through the primary root xylem and its relation to wilting may well be illustrated in watermelon plants where the runners have rooted some distance from the crown. In such cases, if the primary root became infected, that part of the runner between the crown and the point of rooting of the runner wilted while the remainder of the runner was unaffected. In other instances, when the adventitious roots became infected, only that part of the runner beyond the adventitious roots wilted. The seriousness of restricted waterflow through plants infected with a vascular pathogene has been demonstrated by Melhus, Muncie and Ho (37), who compared the rate of water movement in healthy cabbage stems with those infected with *Fusarium conglutinans*. Gum-like materials have been shown by LeClerg and Durrell (30) to interfere with the passage of water in alfalfa roots. Judged from the observations made in the present study of watermelon plants infected with *Fusarium niveum*, it would seem that wilting and collapse of the host are due, wholly or partly, to the lack of movement of

water through the xylem vessels which have become gorged with tyloses, gum-like materials and mycelium.

Toxins and metabolic products elaborated by vascular fusarial pathogenes have been frequently credited with causing wilting and inflicting the injuries manifested by the afflicted host. Support of the theory of toxin effect has been sought in attempts to simulate external pathological symptoms in the host comparable to those present when infected by the pathogene. In certain instances products such as alcohols (2) (3), amine-like substances (1) (41) (58), nitrites (55), carbon dioxide (68), and other materials (21) (28) have been isolated from the filtrates of cultures and the mycelium of fusarial pathogenes. External symptoms have been used too often as criteria for interpreting the effects upon the host by these various materials. Hursh (24), after studying the reaction of plant stems to fungous products, calls attention to the need for a more serious study of the particular cells and tissues directly affected. In the case of watermelon plants, infected with *Fusarium niveum*, direct injury by the presence of the pathogene was manifested in the xylem vessels, where gum-like materials and tyloses were formed by living cells of the host presumably injured by either toxic, enzymatic, or metabolic products of the wilt pathogene and to which might be added the possibility of additional injury to healthy host cells from cells previously injured by the pathogene.

There are indications that the gum-like materials and tyloses, present in the xylem of diseased plants may have a deleterious effect on the pathogene as well as on the host. Fahmy (13) in studying cotton wilt and Linford (38) in the study of pea wilt have intimated that gum-like materials in the vessels may serve to impede the progress of the pathogene in the host. Yoshii (77) has suggested that the formation of tyloses may have a part in obstructing the movement of *Fusarium niveum* within affected vessels of infected watermelon roots. It has been observed in the present study that the end walls of tracheal cells in resistant seedlings become coated with gum-like materials in advance of the invading hyphae of the wilt pathogene (fig. 5, B). It is also significant that when transverse sections were cut from the primary root of living resistant plants, grown in soil heavily infested with the wilt pathogene, a band of gum-like material frequently surrounded the older xylem while the younger secondary xylem near the periphery of the root axis was unaffected. Apparently this deposition of gum-like material coincided with cessation of the pathogene's progress. All of the xylem vessels in the primary roots of wilting susceptible plants were filled with gum-like material. Neither resistant nor suceptible plants grown in soil free of the wilt pathogene

had an appreciable amount of gum-like material and tyloses in their xylem vessels.

Older resistant plants seemingly withstood attacks of the wilt pathogene which in seedlings proved fatal. Apparently no defense mechanism had time to develop sufficiently to function in the early seedling stage such as seems to be developed in older plants. This characteristic reaction in resistant plants appeared consistently in all 4 years of field studies (figs. 9, 10, 11, 12). The heaviest mortality occurred within the first 30 days after planting, following which there was an abrupt decline in the percentage of wilting plants. This abrupt decrease in percentage of wilting plants did not occur in the susceptible variety, Kleckley Sweet, used as a check. The percentage average daily wilt reached a maximum at 16 to 24 days after resistant seedlings were planted, following which the percentage dropped to less than 1 percent in plants 40 to 45 days old. Susceptible seedlings showed a maximum percentage average daily wilt at 23 to 39 days, and the decline in the daily rate of wilt did not drop to as low a level. This reaction to wilting seemed to be correlated directly with the age of the seedlings rather than to the result of environmental factors, as shown in fig. 12, where two groups of seedlings, planted 21 days apart, varied in the incidence of their maximum percentage average daily wilt when grown in the same field and subjected to like environmental conditions.

The effect of environmental factors upon the infectivity of the pathogene has been studied in many of the vascular-fusarial diseases (7) (8) (17) (50) (69) (79). Porter and Melhus (53) found that when certain periods were considered, high air temperatures accompanied by low humidity increased the average daily wilt of watermelon plants grown in fields heavily infested with *Fusarium niveum*. Porter (50) in subsequent field studies in California sustained the earlier results. An interesting point appeared in the later study by Porter (50) which he did not mention, namely the percentage average daily wilt when graphically plotted over the 30 days that readings were taken showed the upward trend which has been observed in the present study of percentage average daily wilt in seedlings of the same age. This upward trend in the percentage average daily wilt in seedlings persisted although temporarily interrupted for 1 or 2 days by variations in air temperature or humidity.

The results in the present greenhouse and field experiments showed resistance to be an inheritable character, manifested in the varieties, Iowa King, Iowa Belle, and Pride of Muscatine. Experiments have been conducted under conditions much more extreme than those prevailing in ordinary infested field soils.

The previous history of the field soils chosen for experimental planting indicated that a heavy infestation of *Fusarium niveum* was present. This infestation became more severe by repeated cropping to watermelons. Successive generations of wilt-resistant varieties and new selections were purposely grown in these fields in order that only the more resistant segregates might survive. The varieties, Iowa King, Pride of Muscatine and Iowa Belle proved to be suitable parental stock for the transmission of resistance when hybridized with susceptible varieties. Jones (26) has also reported these varieties to be desirable for transmitting resistance when hybridized with varieties susceptible to *Fusarium niveum* in California. In the present breeding operations it has been found that a greater resistance was secured by backcrossing the F_1 hybrid, of the susceptible x resistant variety, to the resistant parent which was then followed by inbreeding the segregates for three to four generations.

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